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Molecular Markers (RAPD-PCR) - Tool to supplement the study on Taxonomy and Phylogeny of Lepidoptera

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ABSTRACT

The total number of species of class Insecta, so far described from the whole world is representing nearly 80% of the total species of the Kingdom Animalia. It is estimated that there are 9, 50,000 described species of insects, and lot of new species are added to this every year. Lepidoptera 2nd largest insect order after Coleoptera constitutes a major fauna (Moths and Butterflies) and comprises many species of economic importance and has cosmopolitan presence. Molecular taxonomy of Lepidoptera in India is now getting importance and lot of work in this field has yet to be done. RAPD-markers can be used to differentiate population of insect species and biotype. RAPD-PCR band patterns are species specific and can be used in rapid diagnosis of species. During the present investigation RAPD-PCR band patterns have been developed by using specific DNA isolation kit and thus have been used to characterize and analyze three moth (Lepidoptera) species of cold deserts (Spiti and Leh) of India.

Key words: RAPD PCR, Band, DNA, Lepidoptera, Moth.

1. INTRODUCTION

The total number of species of class Insecta, so far described from the whole world is representing nearly 80% of the total species of the Kingdom Animalia. It is estimated that there are 9, 50,000 described species of insects, and lot of new species are added to this every year. Lepidoptera is 2nd largest insect order after Coleoptera which constitutes a major fauna (Moths and Butterflies) and comprises many species of economic importance and has cosmopolitan presence. Butterflies are particularly sensitive to climate and are important indicators of environmental changes R.L.H. Dennis (1993); Margaret (2008). They are good biological indicators of environmental variation as they are easily noticed as they are diurnal, flying around during sunshine, attractive, and conspicuous, more easily identified

groups compared to others. They are also good biological indicators of environmental quality as they are sensitive and directly affected by any alteration in their habitats, atmosphere, local weather and temperature and micro-climate Watt et al. (1968); Ehrlich et al. (1972); Heath (1981); Rosenberg et al. (1987); Dennis (1993). Molecular taxonomy of Lepidoptera in India is now getting importance and lot of work in this field has yet to be done. RAPD-markers can be used to differentiate population of insect species and biotype.

In recent years DNA profiling through RAPD-technique has been used for the analysis of diversity and identification of duplicates within the large germplasm population Virk *et al*. (1995), phylogenetic relationship Millan *et al*. (1996) and Management of genetic resources Bretting and Widerelechner (1995). Molecular techniques based on DNA sequence polymorphism are now used in population genetics studies, systematic and molecular taxonomy to get answer to systematic related problems Nagaraja and Nagaraju (1995); Tom et al. (1995); Weng et al. (1996); Zhou et al. (2000); Zakharov (2001); Sharma et al. (2003). Evidently, RAPD technology is reproducible, rapid and sensitive technique, which can be used for examination of genomic variation and to estimate relationships between closely and more distantly, related species and groups of insects Sharma et al. (2010). In India Sharma et al. (2006) had done various studies on different butterfly species by RAPD technique. Lepidopteron are of great economic importance especially in their larval stages. This technique is cost effective and less time consuming and easily and reproducible, the results can directly be inferred from the gel and reveals large amount of genetic variations, so it find various entomological applications Hunt and Page (1992); Heckel et al. (1995); Dowdy and Mc- Gaughey (1996).

2. MATERIALS AND METHODS

2.1. DNA isolation

The moth samples were collected from illuminated white sheet from cold desert areas of Leh and Spiti. The samples collected were killed and kept in alcohol preserved in icepacks. Then these moth samples were processed extraction and amplification for further study. DNA isolation was done by Standard Kit i.e. Dneasy Blood &Tissue kit by Qiagen. For DNA isolation adult individuals of both the sexes of each species were used. Single adult individual was processed as per the protocol of the kit with minor modifications. The concentration of DNA was determined by spectrophotometric method using UV visible scanning spectrophotometer.

2.2. DNA Amplification

The DNA was amplified by using three decanucleotide primers with random sequences procured from Genetix (USA) as given in Table 1. Each reaction mixture of 25µl consisted of 2.5µl of 10X PCR buffer, 1µl MgCl2 (2.5Mm), 0.5µl dNTPs (2mM), 1.3 µl of Taq Polymerase (1U/µl), 2.5µl of BSA (100pM /µl), 2.5µl of primer (10µM), 2.5µl of DNA and the rest distilled water. The amplification was carried out in thermal-cycler (MJ Research, USA) under the following PCR conditions (Table 2). The amplified products were run on 2% agarose gel (stained with ethedium bromide) with DNA ladder (100-3000 bp). Gels were photographed under UV illumination.

2.3. Dendrogram Plot

In the present research work clustering method of complete linkage was used for creating a tree of sample similarity. Dendrograms for the two species *i.e. C. crocale* and *C. pyranthe* with three primers were obtained with the help of Quantity One Software. RAPD-PCR band patterns are species specific and can be used in rapid diagnosis of species. During the present investigation RAPD-PCR band patterns have been developed by using specific DNA isolation kit and thus have been used to characterize and analyze three moth (Lepidoptera) species of cold deserts (Spiti and Leh) of India.

The banding patterns observed from DNA extracts with three different noctoids species viz. *Athelis Delecta, Spirama Retorta and Cerora Litura* were compared. Then a particular study with the same molecular tools was performed for the differentiation between the male and female strains in the *Athelis Delecta* spp. The repeatability of the banding patterns was tested on extracted DNA from samples of whole DNA procured from the different strains of moths and also generation of the band patterns in the same strains i.e. extracted DNA from individuals of the same species in. The differences observed in band profiles of the different species were highly significant and enabled the easy differentiation between them. It was shown frequent dissimilarities between the band profiles of strains of. *Athelis Delecta, Spirama Retorta and Cerora Litura.* Male and female strains of *Athelis Delecta* also observed the difference in band profiling. The potential for RAPD-PCR technique to provide useful genetic data for the discrimination up to the inter-specific and intra-specific level in the species of moths found in cold deserts of Spiti (H.P) and Leh (J&K) was of concern and is hereby discussed.

3. RESULTS & DISCUSSION

Three different species were studied for establishing the interspecific and intra specific difference among the same/different species. Each species has difference in pattern of band in agarose gel electrophoresis (Figure 1)

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showing clear discrimination with another species. From the plates we can conclude that in the plate 1, the electrophoresis product (Plate-1a) showed the difference in base pair between the different species (interspecific) as visualized from the above *Athelis Delecta* (m) in lane 1 showed five bands with base pair ranging from 352-372 while molecular weight ranging from 415-612 kd whereas *Spirama Retorta* (m) in lane 5 showed twelve bands with base pair ranging from 276-324 while molecular weight ranging from 896-1396 kd, similarly *Cerura Litura* (f) in lane 6 showed thirteen bands with base pair ranging from 282-357 while molecular weight ranging from 563-133 kd. This plate proves the interspecific difference between the different species at molecular level (Graph 1 & Table 2).

From the same plate it can be adjudged that the difference in base pair between the same species (intraspecific) differing only in gender male(m) and female(f) as visualized from the above (Table 2). *Athelis Delecta* (m) in lane 1&4 showed five bands in common with base pair ranging from 348-372 while molecular weight ranging from 356-681 kd whereas *Athelis Delecta* (f)in lane 3 showed only four bands with base pair ranging from 314-349 while molecular weight ranging from 642-1000 kd. This plate proves the intraspecific difference between the same species at molecular level.

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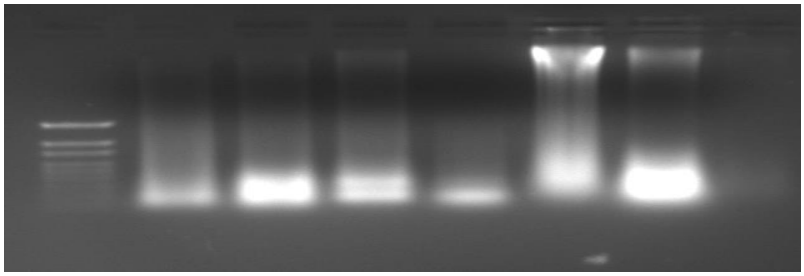


Figure 1

Plate-1a: Agarose gel run for PCR product of 3 Species viz.

Lane 0: 100bp marker

Lane 1: *Athelis Delecta* (m)

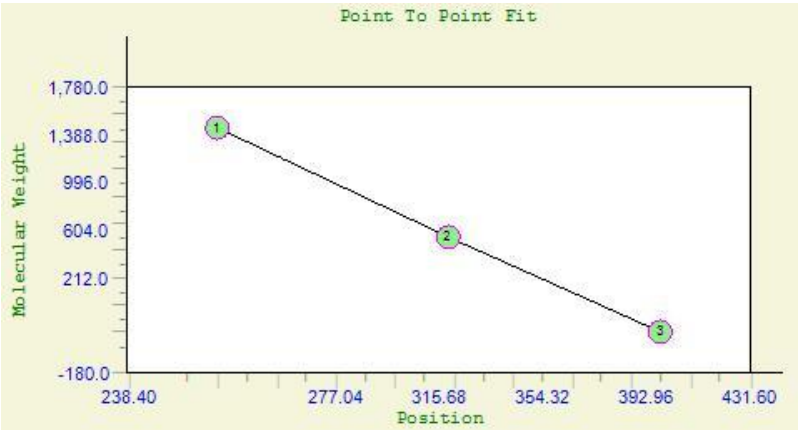
Lane 2: *Athelis Delecta* (f)

Lane 3: *Athelis Delecta* (f)

Lane 4: *Athelis Delecta* (m)

Lane 5: *Spirama Retorta* (m)

Lane 6: *Cerora Litura* (f)



Graph 1

Base pair difference, molecular weight analysis and rf value determination of the marker and species differentiation

Table 1

Primer	Sequence5'->3'	Tm Value OC	GC Content %
OPA1	CAGGCCCTTC	34	70
OPA2	TGCCGAGCTG	34	70
OPA3	AGTCAGCCAC	32	60
OPA4	AATCGGGCTG	32	60
OPA5	AGGGGTCTTG	32	60
OPA6	GGTCCCTGAC	34	70
OPA7	GAAACGGGTG	32	60
OPA8	GTGACGTAGG	32	60
OPA9	GGGTAACGCC	34	70
OPA10	GTGATCGCAG	32	60
OPA11	CAATCGCCGT	32	60
OPA12	TCGGCGATAG	32	60
OPA13	CAGCACCCAC	34	70
OPA14	TCTGTGCTGGC	32	60

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OPA15	TTCCGAACCC	32	60
OPA16	AGCCAGCGAA	32	60
OPA17	GACCGCTTGT	32	60
OPA18	AGGTGACCGT	32	60
OPA19	CAAACGTCGG	32	60
OPA20	GTTGCGATCC	32	60

Table 2

Marker

Lane	Band	Position	Mol. Weight	Rf
0	1	266	1500	0.346
0	2	338	750	0.44
0	3	404	100	0.526

Species base pair difference

Lane	Band	Position	Mol. Weight	Rf
1	1	352	612	0.458
1	2	356	573	0.464
1	3	360	533	0.469
1	4	368	455	0.479
1	5	372	415	0.484
2	1	325	885	0.423
2	2	337	760	0.439
2	3	349	642	0.454
2	4	354	592	0.461
2	5	365	484	0.475
3	1	314	1000	0.409
3	2	333	802	0.434
3	3	340	730	0.443
3	4	349	642	0.454
4	1	345	681	0.449
4	2	350	632	0.456
4	3	357	563	0.465
4	4	369	445	0.48
4	5	378	356	0.492
5	1	276	1396	0.359
5	2	278	1375	0.362
5	3	285	1302	0.371
5	4	290	1250	0.378
5	5	296	1188	0.385
5	6	300	1146	0.391
5	7	306	1083	0.398
5	8	309	1052	0.402
5	9	312	1021	0.406
5	10	314	1000	0.409
5	11	320	938	0.417

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5	12	324	896	0.422
6	1	282	1333	0.367
6	2	292	1229	0.38
6	3	296	1188	0.385
6	4	308	1062	0.401
6	5	316	979	0.411
6	6	322	917	0.419
6	7	329	844	0.428
6	8	334	792	0.435
6	9	338	750	0.44
6	10	341	720	0.444
6	11	346	671	0.451
6	12	352	612	0.458
6	13	357	563	0.465

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